Pretreatment with a Water-Based Surfactant Formulation Affects Transdermal Iontophoretic Delivery of R-Apomorphine *in Vitro*

Gai Ling Li,[1] Meindert Danhof,[2] Peter M. Frederik,[3] and Joke A. Bouwstra[1,4]

Received October 30, 2002; accepted January 2, 2003

Purpose. To further increase the transdermal transport rate of R-apomorphine, a nonocclusive pretreatment with an aqueous surfactant formulation in combination with iontophoresis was explored *in vitro*.

Methods. The human stratum corneum was pretreated nonocclusively with formulations composed of laureth-3 oxyethylene ether ($C_{12}EO_3$), laureth-7 oxyethylene ether ($C_{12}EO_7$), and cholesterol sulfate (CSO_4) prior to iontophoresis. The effect on the flux of the following parameters was examined: the composition, the charge, and the applied amount of surfactant formulations.

Results. The iontophoretic flux of R-apomorphine was appreciably increased by pretreatment with surfactant formulations. A formulation containing $C_{12}EO_3/C_{12}EO_7/CSO_4$ at a molar ratio of 70:30:5 was very stable and increased the iontophoretic flux of R-apomorphine from 92.2 ± 13.9 nmol/cm^2*h to 181.5 ± 22.6 nmol/cm^2*h. When further increasing the negative charge of this formulation the iontophoretic transport rate was slightly inhibited. A dose of 40 μL/cm^2 of the formulation with a total surfactant concentration of 5% (w/w) was sufficient for a maximum enhancing effect.

Conclusions. The results obviously show that nonocclusive pretreatment with the surfactant formulation enhances the iontophoretic transport of R-apomorphine, and is a promising approach to achieve therapeutic concentrations of R-apomorphine.

KEY WORDS: iontophoresis; R-apomorphine; non-occlusive pretreatment; surfactant formulation.

INTRODUCTION

R-apomorphine, the first dopamine agonist to be synthesized, has received a considerable interest again since the late 1980s, especially because of its utility in the treatment of motor fluctuations in patients with Parkinson's disease. This compound is the most effective dopamine agonist currently available but is difficult to administer because of a strong first-pass effect upon oral administration and its inherent instability (1,2). Therefore, several alternative administration routes for R-apomorphine have been investigated (2). One of the alternative routes is transdermal administration. In general, transdermal iontophoresis has been shown useful for the delivery drugs into human bloodstream at a continuous and well-controlled rate (3,4). Thus potentially it offers the opportunity to circumvent the problems associated with conventional modalities for R-apomorphine delivery.

The feasibility of delivery of R-apomorphine by iontophoresis has been demonstrated in the previous *in vitro* and *in vivo* studies (5,6). The results of these studies indicate that the input rate of R-apomorphine into the bloodstream can be substantially enhanced by transdermal iontophoresis. In addition, it was shown that the delivery rate of R-apomorphine can be accurately controlled by modulating the applied current density. Furthermore, skin irritation resulting from the electric field was at a clinically acceptable level. Thus transdermal iontophoresis allows in theory a well-controlled and individualized delivery of R-apomorphine. Nevertheless, due to the skin barrier function, the extrapolated R-apomorphine steady-state plasma concentrations in most patients were only at low limits of the therapeutical range after 1-h iontophoresis at a current density of 375 μA/cm^2.

An important question is therefore how the transdermal transport rate of R-apomorphine can be enhanced further while maintaining the accurate and individualized control of the delivery. At present there is considerable interest in the combination of chemical enhancers with iontophoresis (7,8). It is expected that a combination of chemical and physical enhancement may allow the use of relatively low quantities of enhancers and current within the delivery system thereby preventing potential adverse reactions, irreversible structural changes to the skin and dermatoxicities. Some studies have demonstrated that there is a synergistic or additive effect between chemical enhancers and iontophoresis. For example, it has been reported that the iontophoretic flux of propranolol hydrochloride was effectively increased two-fold after the pretreatment with sodium lauryl sulfate (9). In contrast, unfavorable nonadditive effects were demonstrated in the transdermal iontophoresis of sotalol and salicylate in the presence of penetration enhancer dodecyl *N*,*N*-dimethyl amino acetate and Azone (10). At present little is known about the underlying mechanism of the transport enhancement. A successful application of this approach is dependent on the understanding of 1) the contribution of each driving force to the overall diffusion rate such as convective flow, electroosmosis and electromigration; 2) the specific transport pathway for a particular drug; and 3) the mechanism of action of an enhancer on the skin permeability.

Recently, we have shown that occlusive pretreatment with laureth-3 oxyethylene ether ($C_{12}EO_3$) dissolved in propylene glycol significantly enhances the iontophoretic transport of R-apomorphine across human stratum corneum (11). The degree of this enhancing effect was dependent on the concentration of $C_{12}EO_3$ and the duration of pretreatment. A pretreatment with 0.30 M $C_{12}EO_3$ for 24 h induced a 3.3-fold increase in the iontophoretic transport rate of R-apomorphine compared to iontophoresis alone, whereas a pretreatment period of 6 h resulted in only a factor 1.3 enhancement. However, this occlusive pretreatment is not very practical, and particularly it may lead to unacceptable skin irritation. For this reason the aim of the present study was to develop a new generation of surfactant formulations that can

[1] Department of Pharmaceutical Technology, Leiden Amsterdam Centre for Drug Research, University of Leiden, P.O. Box 9502, 2300 RA, Leiden, The Netherlands.

[2] Department of Pharmacology, Leiden Amsterdam Centre for Drug Research, University of Leiden, P.O. Box 9502, 2300 RA, Leiden, The Netherlands.

[3] Department of Pathology, University of Limburg, P.O. Box 616, 6200 MD, Maastricht, The Netherlands.

[4] To whom correspondence should be addressed. (e-mail: Bouwstra@chem.leidenuniv.nl)

0724-8741/03/0400-0653/0

be applied nonocclusively in the absence of organic solvents, but which contain $C_{12}EO_3$ to retain the penetration enhancing effect for R-apomorphine transport. In order to select the most effective formulation for enhancement during iontophoresis, the composition, the applied amount and the charge of surfactant formulations were varied.

MATERIALS AND METHODS

Materials

The non-ionic surfactant laureth-3 oxyethylene ether ($C_{12}EO_3$) and laureth-7 oxyethylene ether ($C_{12}EO_7$) were gifts from Servo (Delden, the Netherlands). Sodium sulfosuccinate was a gift from Cytec Industries (Rotterdam, The Netherlands). Cholesterol sulfate (CSO_4) was purchased from Sigma Chemicals (Hilversum, The Netherlands). R-apomorphine hydrochloride was obtained from OPG (Utrecht, the Netherlands). Its purity was tested by high-performance liquid 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, UK) was used as a solvent in the HPLC analysis. Dialysis membrane disks (cut-off value: 5000 Da) were obtained from Diachema (München, Germany). Trypsin (Type III; from a bovine pancreas) and trypsin inhibitor (Type II-S from soybean) were purchased from Sigma Chemicals (Zwijndrecht, The Netherlands). All other chemicals used were of analytical grade. Millipore water (resistivity $\geq$18 MΩ) was used to prepare all solutions.

Preparation and Characterization of Surfactant Formulations

The composition of the surfactant formulations is listed in Table I. The formulations were prepared by a modification of the sonication method described by Baillie *et al.* (12). Briefly the surfactants were dissolved in ethanol. The solvent was evaporated overnight in a vacuum centrifuge and the remaining surfactant film was hydrated with phosphate-buffered saline (PBS). Furthermore, the formulations were sonicated for 15 s at room temperature. The sonicator used was a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, CT, USA) with a 1/8-inch microtip at a 60-watt energy output.

The surfactant formulations were pre-examined for size distribution, phase separation, and stability at room temperature for at least 1 week. The z-average diameters of the particles in surfactant formulation were determined by Dynamic light scattering using a Malvern 4700 with a 25 mW He-Ne laser (Malvern Ltd., Malvern, UK) and the Automeasure software (version 3.2). The samples were diluted in order to avoid multiple scattering. The polydispersity index, which is a measurement of formulation homogeneity, ranged from 0.0 (monodisperse) to 1.0 (very heterogeneous). The temperature was set at 27°C.

The surfactant formulations prepared from $C_{12}EO_3$, and from $C_{12}EO_3$, $C_{12}EO_7$, CSO_4 in molar ratio of 70:30:5 (total lipid 5% w/w) were visualized by cryo-transmission electron microscopy (cryo-TEM). The technique of cryo-TEM has been described in detail by Frederik *et al.* (13). Briefly, a grid (hexagonal pattern, 700 mesh) was dipped and withdrawn from the surfactant formulation. After withdrawing, excess formulation was blotted away with filter paper. This method resulted in the formation of thin sample films spanning the grid holes (30 μm$\varnothing$). These films were vitrified by plunging the grid into liquid ethane. The vitrified formulation film was transferred into a Philips CM12 microscope (120 KV) using a Gatan cryotransfer system (Philips, The Netherlands) and visualised at a temperature between –170°C and –173°C in conjunction with a low-dose imaging device.

Nonocclusive Pretreatment with Surfactant Formulation

Human abdomen skin was obtained after cosmetic surgery. Isolation of human stratum corneum (SC) was carried out as described previously (5). SC was stored above silica gel in dark. Before use circular samples of 18mm diameter were punched and hydrated for 1 h by floating them with the dermal side on a PBS solution at pH 7.4. Subsequently the circular pieces of SC were placed in Franze-type diffusion cells with the SC side facing the donor compartment. Dialysis membrane disks (cut-off value: 5000 Da) were placed underneath the SC to serve as supporting membrane. The receiver compartment was filled with PBS pH 7.4 and heated to 32°C. Care was taken to prevent the formation of air bubbles between the dermal side of skin and the receiver solution.

After the mounting of the skin, 40 μL of each surfactant formulation listed in Table I was applied to the skin, except for one series of experiments in which 30, 60, and 80 μL were used. The SC was treated under nonocclusive conditions for 3 h with freshly prepared surfactant formulations. For

Table I. Composition of Surfactant Formulation in Phosphate-Buffered Saline and Characterization

Preparations	Total percent surfactant concentration (w/w)	Composition (mol %)	Phase behavior	Dynamic light scattering results	
				Particle size (nm)	Polydispersity
$C_{12}EO_3$:$C_{12}EO_7$:CSO_4	5	100:0:0	+	137 ± 23	0.28
		90:10:5	+	83 ± 2	0.40
		70:30:5	–	70 ± 8	0.26
		50:50:5	+	144 ± 7	0.25
$C_{12}EO_3$:$C_{12}EO_7$:CSO_4	8.9	70:30:5	–	73 ± 21	0.18
	15	70:30:5	–[v]	74 ± 6	0.16
$C_{12}EO_3$:$C_{12}EO_7$:Sulfosuccinate	5	70:30:10	–	65 ± 7	0.23

Note: + indicates that phase separation occurred; – means that no phase separation was observed; V indicates that the formulation was viscous and gel-like.

comparison SC treated with 40 μL of PBS buffer solution served as the control.

In Vitro Iontophoresis Study

A nine-channel computer controlled power supply was used to provide a constant current (Central Electronics Department, Gorlaeus Laboratories, Leiden University, The Netherlands). This instrument is also capable of measuring the resistance across the skin while current supply is on. A silver plate electrode was used as an anode, a silver/silver chloride electrode as a cathode. All diffusion experiments were carried out in three chamber continuous flow-though diffusion cells. The area of skin exposed to the electrode compartment was 0.64 cm^2.

After pretreatment the SC was transferred to the iontophoretic set-up, and placed between the donor and intermediate chamber with the SC facing the donor compartment. Between the intermediate and acceptor chamber there was a second piece of non-pretreated SC. At least three skin specimens were used for every experimental condition studied. Dialysis membrane (cut-off, 5000 Da) was used as a supporting membrane. In the actual experiment, 15 mM R-apomorphine hydrochloride dissolved in 5 mM citrate buffer (containing 8 g/L NaCl and 1 g/L sodium meta bisulphite**,** pH 5.0) was applied in the anodal chamber. The receiver chamber was filled with PBS, pH 7.4. The intermediate chamber was continuously perfused using a peristaltic pump with PBS buffer, pH 7.4, at a rate of 7 mL/h. Samples were collected hourly with a fraction collector. During the experiments both the anodal and the cathodal chambers were magnetically stirred at 375 rpm. To prevent oxidative breakdown of R-apomorphine the fraction collector was kept in a covered box with a N_2 gas flow through system. In addition, 200 μL of antioxidant solution (0.05% EDTA, 0.5% sodium meta bisulphite dissolved in 25% H_3PO_4) was added in each collecting tube. No degradation of R-apomorphine in diffusion samples was detected by HPLC analysis. All experiments were conducted at a constant current density of 500 $\mu A/cm^2$. The total permeability experiment included three stages: 6-h passive diffusion, 9-h iontophoresis, and 5-h post-iontophoresis passive diffusion.

Analytical Methods

Diffusion samples were injected directly into a HPLC system consisting of a SP8810 LC pump (Spectra-Physics, Inc., CA, USA), a Gilson 234 autoinjector and a fluorescence detector (Jasco 821-FP, H.I. Ambacht, The Netherlands). A nucleosil 100, 5-μm C-18 column was used (200 mm × 4.6 mm I.D.). The mobile phase consisted of acetonitrile/0.1 M phosphate buffer (25/75 v/v). The phosphate buffer of pH 3 contained 0.1 M NaH_2PO_4, 20 mg/mL 1-octanesulfonic acid and 10 mg/mL EDTA. Detection was at an excitation wavelength of 280 nm and an emission wavelength of 460nm (Gain 10 and attenuation 1). The injection volume was 20 μL. Freshly made standard solutions were used to get calibration curves for each experiment. The calibration curves were linear (r > 0.999) in the concentration range of 125–2500 ng/mL. The intra- and inter-assay variations were <5% for all concentrations tested. The detection limit under these conditions was 10 ng/mL.

Data Analysis

The cumulative amount of R-apomorphine permeated per unit skin area was plotted against time. The slope of the linear portion of this plot was estimated as the steady-state flux, whereas the lag time was calculated from the intercept in x-axis. The enhancement ratio ER was calculated by the iontophoretic steady-state flux across surfactant treated skin divided by the iontophoretic steady-state flux across PBS-treated skin. Results were presented as mean values ± standard deviations. One-way analysis of variance at a level of significance $p < 0.05$, was used to compare data sets.

RESULTS

Property of Surfactant Formulations

We have examined various compositions of surfactant formulations prepared from $C_{12}EO_3$, $C_{12}EO_7$ and cholesterol sulfate. Their particle sizes and corresponding polydispersities as measured by dynamic light scattering, are summarized in Table I together with the physical stability as observed by visual inspection. Furthermore, two surfactant formulations, one containing 5% (w/w) $C_{12}EO_3$ only and another one containing $C_{12}EO_3$, $C_{12}EO_7$, and CSO_4 in molar ratio of 70:30:5, were visualized by cryo-TEM and their representative micrographs are given in Fig. 1A and B, respectively.

The particle sizes of the freshly made surfactant formulations are all in the range between 65 nm and 144 nm (shown in Table I). During storage aggregation or fusion was observed resulting in phase separation in all formulations except for those containing $C_{12}EO_3$:$C_{12}EO_7$ in a molar ratio of 70:30. Of particular interest is the observation that the formulation prepared from $C_{12}EO_3$:$C_{12}EO_7$: CSO_4 in the molar ratio of 70:30:5 behaved remarkably different from the others. This formulation was fairly stable at room temperature for at least 6 month with respect to particle size and phase separation.

Fig. 1A shows that the $C_{12}EO_3$ formulation mainly contains unilamellar vesicles and some vesicles show perforated bilayers. In Fig. 1B, it is shown that when 30% $C_{12}EO_7$, a micelle forming surfactant, is incorporated in the formulation, only large thread-like micelles are observed. Therefore it is reasonable to deduce that the formulations with an intermediate $C_{12}EO_3/C_{12}EO_7$ molar ratio are presumably mixtures of large bilayer fragments and micelles.

Effect of Surfactant Formulations on R-Apomorphine Transport

A series of surfactant formulations (5% w/w) was prepared in which the molar ratio of $C_{12}EO_3/C_{12}EO_7/CSO_4$ varied from100:0:0 to 90:10:5, 70:30:5 and 50:50:5. To further increase the negative charge of the formulation, the negatively charged component was increased from 5 to 10% (w/w).

An example of the diffusion profile of R-apomorphine across human SC after pretreatment with either $C_{12}EO_3/C_{12}EO_7/CSO_4$ = 70:30:5 surfactant formulation or PBS is shown in Fig. 2. Similar permeation profiles were also observed with the other surfactant formulations examined. As shown in Fig. 2, during 6 h pre-iontophoresis passive diffusion, the amount of R-apomorphine in the receptor chamber was below the detection limit of the HPLC method. After switching on the current, the R-apomorphine flux was dra-

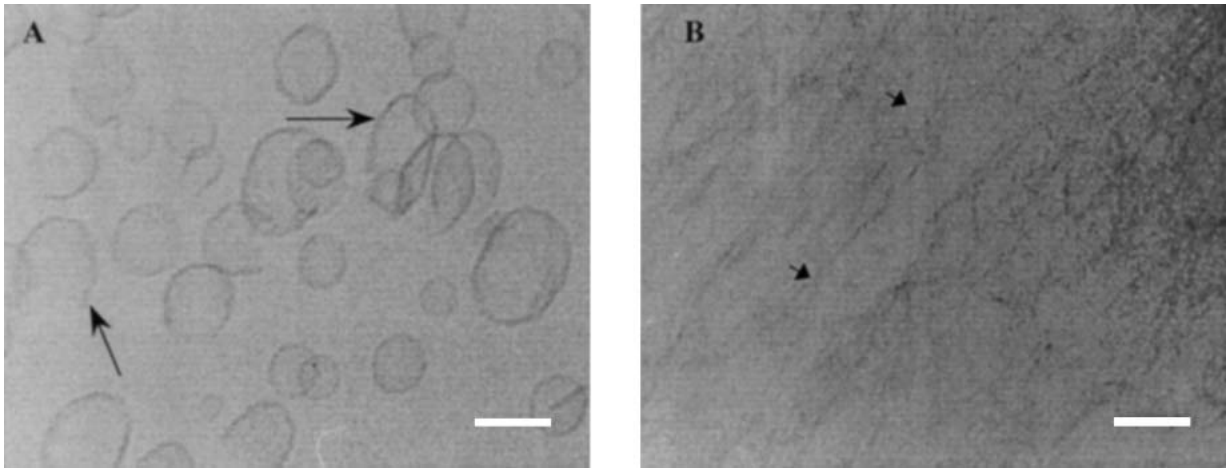

Fig. 1. Micrographs of surfactant formulations (5% w/w total surfactant) visualized by cryo-transmission electron microscopy. (A) $C_{12}EO_3$ only; (B) $C_{12}EO_3$:$C_{12}EO_7$:CSO_4 = 70:30:5 (in molar ratio). Scale bar represents 50 nm. Big Stealth arrow = perforated bilayer; Small arrow = thread-like micelle.

matically increased and reached a steady-state situation within a short period. Upon termination of the electric current application, the flux decreased to a final post-iontophoretic plateau that was higher than the pre-iontophoresis passive level. Table II summarizes the calculated steady-state fluxes for each experimental interval and their corresponding enhancement ratios.

Compared with control (PBS), the iontophoretic steady-state fluxes and the post-iontophoresis passive fluxes were substantially increased ($p < 0.01$) by pretreatment with all surfactant formulations tested, whereas the pre-iontophoresis passive fluxes were still not detectable. Varying $C_{12}EO_3$: $C_{12}EO_7$:CSO_4 molar ratio from 100:0:0 to 70:30:5 did not significantly change the iontophoretic R-apomorphine transport ($p > 0.05$). However, a further change to 50:50:5 induced a significant decrease ($p<0.01$) in R-apomorphine iontophoretic steady-state flux from 181 ± 23 nmol/cm^2*h to 138 ± 19 nmol/cm^2*h. Moreover, increasing the negative charge of the formulation did not further increase the permeation of R-apomorphine, but rather seemed to decrease the transport ($p < 0.05$ compared with the control). Based on these results, the composition of surfactant formulation containing $C_{12}EO_3$/$C_{12}EO_7$/CSO_4 p:30:5 was used in the follow-up of this study.

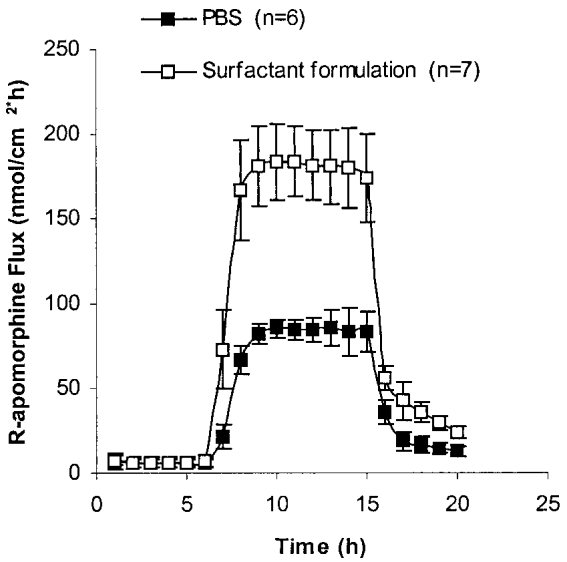

Fig. 2. The permeation profile of R-apomorphine across human SC during 6-h passive diffusion, 9-h iontophoresis at a current density of 500 μA/cm^2, and 5-h postiontophoresis passive diffusion. (□) pretreatment with 40μl of the surfactant formulation composed of $C_{12}EO_3$: $C_{12}EO_7$: CSO_4 =70: 30: 5; (■) pretreatment with 40 μL of phosphate-buffered saline.

Effect of Applied Volume and Concentration of Surfactant Formulation on R-Apomorphine Transport

The amount of surfactant applied on the skin was varied in two different ways. First, the surfactant amount was increased by changing the applied volume of the surfactant formulation at a concentration of 5% (w/w) from 30 to 80 μL/cm^2. Thirty microliters was chosen as a minimum volume, which was able to completely cover the skin surface exposed to iontophoresis. The data in Fig.3A show that 40 μL/cm^2 is the least amount of the formulation for achieving the maximal iontophoretic steady–state flux of R-apomorphine and thereby is considered as the optimal volume.

Second, the concentration of surfactant was increased from 5%, to 8.9%, and 15% while keeping the applied volume of surfactant formulation at 40 μL/cm^2. These results are shown in Fig. 3B. For the surfactant concentration of 8.9%, the observed transdermal iontophoretic flux was 187 ± 35 nmol/cm^2*h, which was not significantly different ($p > 0.05$) from the value for the 5% formulation (182 ± 23 nmol/cm^2*h). However, a further increase in surfactant concentration to 15% reduced the iontophoretic flux of R-apomorphine significantly ($p < 0.05$), possibly due to an increase in the viscosity of the formulation (see Table I).

Effect of Surfactant Formulation on the Electric Property of the Skin

During the diffusion experiments, the resistance data were collected by the iontophoresis set-up. Figure 4 shows the profile of the skin's resistance during 9 h of iontophoresis following the different pretreatments employed. For the control, current passage led to a significant and rapid decrease in

Table II. The Effect of the Nonocclusive Pretreatment on the Iontophoretic Delivery of R-Apomorphine across Human Stratum Corneum because of Various Composition Changes in the Surfactant Formulation (n = 6~9)

Composition of surfactant formulation (in molar ratio)	Iontophoretic steady-state flux (nmol/cm^2 * h)	Enhancement ratio[c]	Post-iontophoresis passive flux (nmol/cm^2 * h)
$C_{12}EO_3$:$C_{12}EO_7$:CSO_4			
100:0:0	195 ± 15[a]	2.1	20 ± 4
90:10:5	177 ± 18[a]	1.9	24 ± 3
70:30:5	181 ± 23[a]	2.0	24 ± 3
50:50:5	138 ± 19[a,b]	1.5	14 ± 7
$C_{12}EO_3$:$C_{12}EO_7$:Sulfosuccinate			
70:30:10	146 ± 30[a,b]	1.6	18 ± 4
Control (phosphate-buffered saline)	92 ± 14	—	10 ± 4

[a] Significant difference over the control ($p < 0.05$).
[b] Significant difference over the composition of 70:30:5 ($p < 0.05$).
[c] Enhancement ratio = [iontophoretic flux with surfactant pretreatment]/[iontophoretic flux with control].

resistance, and then remained constant at this diminished level. However nonocclusive pretreatment with the surfactant formulation dramatically reduced the skin's electric barrier even before the application of current. Once current passage had commenced, skin resistance in this case had decreased to 66% of the pretreatment value. The data show that pretreatment with the surfactant formulation considerably reduced the resistance of human SC compared to the control ($p < 0.01$).

DISCUSSION

In the previous studies, an impressive enhancement of the iontophoretic delivery of R-apomorphine across human stratum corneum was achieved after the occlusive pretreatment with a non-ionic surfactant $C_{12}EO_3$ dissolved in propylene glycol (11). This result urged us to seek for a better enhancer formulation in order to be successful and minimize side effects *in vivo*.

Initially we prepared a 5% (w/w) $C_{12}EO_3$ formulation in normal PBS by a sonication method. Although a remarkable enhancing effect was obtained (see Table II), aggregation and phase separation occurred in this formulation during storage (see Table I). Therefore, there was a need for increasing the stability of the formulation. To achieve this, we decided to add $C_{12}EO_7$ and CSO_4. These amphiphiles increase the steric repulsion ($C_{12}EO_7$) and introduce electrostatic repulsion (CSO_4) between the small aggregates, which is expected to stabilize the formulation. Furthermore due to the intercalation of a micelle forming surfactant $C_{12}EO_7$ the vesicle formulation (see Fig. 2A) turned into a formulation with large micelles (see Fig. 2B).

As demonstrated in Table II, the enhancement of the non-occlusive surfactant formulation pretreatment on the iontophoretic transport of R-apomorphine was affected by the composition of formulations. Among all the tested formulations, the formulation composed of $C_{12}EO_3$:$C_{12}EO_7$:CSO_4 at molar ratio of 70:30:5 was the most effective and the most stable formulation. This suggests that $C_{12}EO_3$ is the key component in the formulation, being more effective than $C_{12}EO_7$ in enhancing the iontophoretic flux of R-apomorphine. Presumably this results from the difference in hydro-

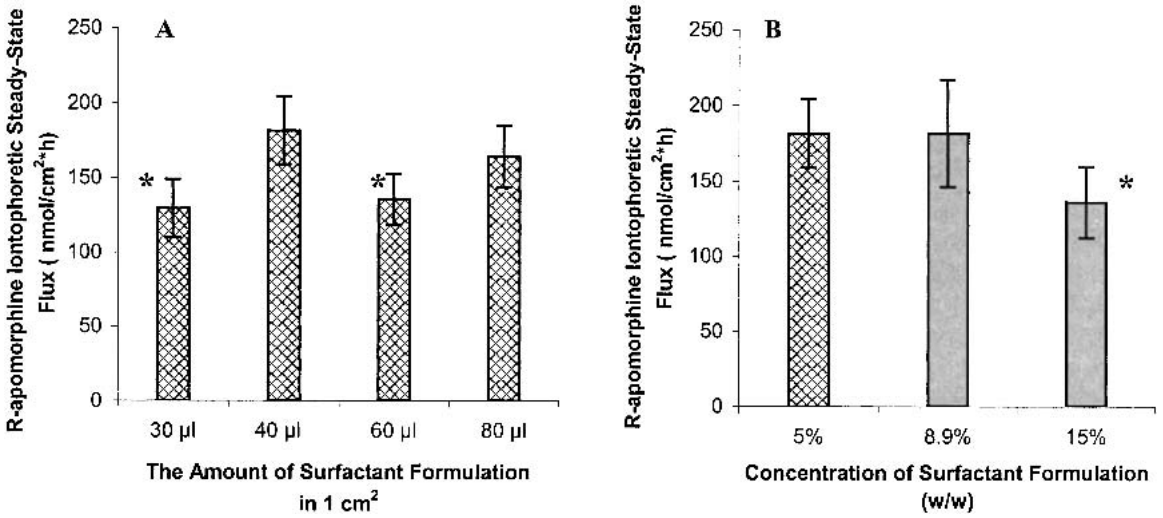

Fig. 3. The effect of surfactant formulation amount applied on the iontophoretic delivery of R-apomorphine across human SC. The surfactant formulation composed of $C_{12}EO_3$:$C_{12}EO_7$:CSO_4 = 70:30:5 in molar ratio. (A) The total surfactant concentration was 5% (w/w). *Indicates significant difference over 40 µL/cm^2 (n = 5~7). (B) The concentration of surfactant formulation was varied from 5 to 15%. The applied volume was kept constant at 40 µL/cm^2. *Indicates significant difference over 5% (n = 6~7).

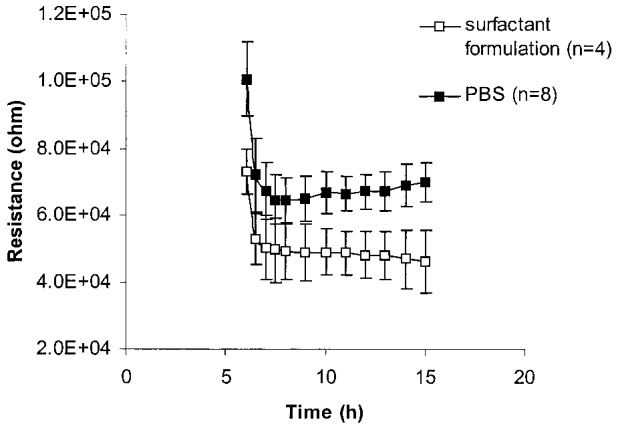

Fig. 4. Resistance of pretreated human SC as a function of time during iontophoresis at a current density of 500 $\mu A/cm^2$. Current is switched on at t = 6 h and switched off at t hour. (■) pretreatment with PBS; (□) pretreatment with 40 μL of the surfactant formulation composed of $C_{12}EO_3$:$C_{12}EO_7$:CSO_4 p70:30:5.

philic-lipophilic balance (HLB) value between these two surfactants. $C_{12}EO_3$ has a smaller HLB value (HLB = 9.3) compared to $C_{12}EO_7$ (HLB = 13.2), which promote the partitioning of the surfactant into the lipid lamellae in the stratum corneum. Similar phenomena have been described for other enhancers in the literature. Aungst (14) has reported that there was a clear relationship between HLB and penetration enhancing effect for non-ionic and anionic surfactants with laureate hydrophobic groups. In these investigations, surfactants with low HLB values were found to have greater effects on naloxone skin penetration.

Extensive investigations have shown that anionic surfactants are found to favor the delivery of positive/neutral compounds from the anodal compartment due to the modification of the surface charge density of the skin (15). For example, adsorption of sodium dodecyl sulfate was found to increase the negative charge of the skin and thereby enhanced the contribution of electroosmosis to the iontophoretic transport of [^{14}C] urea and [^{3}H] sucrose (16). Accordingly the overall iontophoretic transport rate of R-apomorphine is expected being enhanced by the increase in the negative charge of the surfactant formulation applied on the skin from 5% to 10%. However, surprisingly the opposite inhibition action was observed in our study. In a previous study it has been reported that R-apomorphine can readily bind to the lipophilic part of phospholipids and proteins (17,18). Therefore an increase in the net negative charge in the skin might unfortunately increase the binding of R-apomorphine to the skin thereby reducing the transport of R-apomorphine.

Among the volumes/concentrations of the applied surfactant formulations examined (see Fig. 3A and 3B), 40 $\mu L/cm^2$ of the surfactant formulation at a concentration of 5% (w/w) was observed to be the optimal. Because the inherent total lipid amount in the human SC is within a range of 20 to 25% (w/w, data unpublished), the amount of surfactant in 40 $\mu L/cm^2$ of surfactant formulation (5% w/w) is already 10 times as the total amount of native lipid in the skin. Therefore, it seems that 40 $\mu L/cm^2$ is mainly required to fully cover the skin surface. A further increase is not necessary because an excess of surfactant is already present.

From our results it can be concluded that nonocclusive pretreatment of the surfactant formulation containing $C_{12}EO_3$ enhances the iontophoretic transport of R-apomorphine significantly. The mechanism of action cannot be deduced directly from the current data. Most probably several mechanisms are involved. First, the hydration force may act as an additional force to drive the surfactant associates into deeper regions of the stratum corneum, similarly to that proposed by Cevc (19) for transfersomes. Second, penetration of surfactants into the skin may modulate the lipid lamellae in the skin. Such changes in the structural properties of human SC might induce a decrease in the microviscosity and an increase in the diffusion coefficient. This, in turn, facilitates the mobility of large solutes under electric field, thereby increasing the fraction of current carried by the drug. A previous study has shown that following the occlusive pretreatment with a $C_{12}EO_3$ containing vesicle formulation the highly ordered structure of the skin intercellular lipid domains are disrupted, and that water pools are formed in between the corneocytes (20). In addition, the present study shows that the increase in iontophoretic flux is also associated with the decreased skin resistance by the surfactant treatment (Fig. 4).

In the literature, only a few studies have attempted to probe the mechanisms of action of iontophoresis in combination with chemical enhancers by using different physical techniques such as electron microscopy (21–23), attenuated total reflectance-Fourier transform infrared spectroscopy, X-ray diffraction and differential scanning calorimetry (9), and impedance spectroscopy measurements (24). In these studies, similar hypotheses were suggested. Currently the effect of the combined electric and chemical enhancement on the SC ultrastructure is examined in our group. This will provide more insights into mechanisms of action of these surfactant formulations.

Overall, our study demonstrates that 3-h nonocclusive pretreatment with the optimal surfactant formulation consisted of $C_{12}EO_3$:$C_{12}EO_7$:CSO_4 at molar ratio of 70:30:5, is an effective and more practical way to enhance the iontophoretic transport of R-apomorphine. Compared with the previous study the enhancement ratio obtained in the present study is very similar to that after 24 h occlusive pretreatment with 0.15 M $C_{12}EO_3$ in propylene glycol, and significantly higher compared to the effect caused by 6 h pretreatment with 0.30M $C_{12}EO_3$ in propylene glycol. This indicates that within a shorter period of pretreatment, this newly developed surfactant formulation works more efficiently. Assuming a similar flux *in vivo* as obtained *in vitro* in term of iontophoretic steady-state flux of R-apomorphine, the steady state plasma concentration of R-apomorphine 7.6 ng/mL (Given 60 kg per person) *in vivo* will be expected, if iontophoresis is applied from a $20cm^2$ patch at a current density of 500 $\mu A/cm^2$. This means the required therapeutic levels may be achieved in Parkinson's patients. With regard to safety aspect, we expect that the newly developed nonocclusive pretreatment is less irritating than the conventional occlusive pretreatment. Previous dermal toxicity studies focussed on the influence of the surfactants on the proliferation of cultured human keratinocytes have provided evidences to support that surfactants in an aqueous form like vesicle formulation appear to be much safer than the same compounds dissolved in organic solvent like propylene glycol (25). Recently the safety of the iontophoretic delivery in combination with the surfactant formulation pretreatment has been investigated in an *in vivo* study in healthy volunteers.

The results show that such a combined delivery system is a safe process with only minor local skin irritations.

In summary, to enhance and control the transdermal iontophoretic delivery of R-apomorphine a non-occlusive pretreatment with the surfactant formulation containing $C_{12}EO_3$ was successfully developed in the present study. The transdermal iontophoretic transport of R-apomorphine was increased by a factor of 2 under the optimal pretreatment conditions, resulting in an estimated therapeutically relevant input rate for the treatment of Parkinson's disease. Because no organic solvent is involved, and a short-term nonocclusive application of pretreatment is adopted, it is very promising to bring this new iontophoresis delivery system of R-apomorphine to the clinical stage of development.

REFERENCES

1. C. Colosimo, M. Merello, and A. Albanese. Clinical usefulness of apomorphine in movement disorders. *Clin. Neuropharmacol.* **17**: 243–259 (1994).
2. D. Muguet, E. Broussolle, and G. Chazot. Apomorphine in patients with Parkinson's disease. *Biomed. Pharmacother.* **49**:197–209 (1995).
3. H. Sage. Iontophoresis. In E. W. Smith and H. I. Maibach (eds.), *Percutaneous Penetration Enhancers*, CRS Press, Boca Raton, Florida, 1995 pp. 351–368.
4. V. Nair, O. Pillai, R. Poduri, and R. Panchagnula. Transdermal iontophoresis. Part I: Basic principles and considerations. *Methods Find. Exp. Clin. Pharmacol.* **21**:139–151 (1999).
5. R. van der Geest, M. Danhof, and H. E. Boddé. Iontophoretic delivery of apomorphine I: *In vitro* optimization and validation. *Pharm. Res.* **14**:1798–1803 (1997).
6. R. van der Geest, T. van Laar, J. Gubbens-Stibbe, H. E. Boddé, and M. Danhof. Iontophoretic delivery of apomorphine II: An *in vivo* study in patients with Parkinson's disease. *Pharm. Res.* **14**: 1804–1810 (1997).
7. S. Mitragotri. Synergistic effect of enhancers for transdermal drug delivery. *Pharm. Res.* **17**:1354–1359 (2000).
8. E. H. Choi, S. H. Lee, S. K. Ahn, and S. M. Hwang. The pretreatment effect of chemical skin penetration enhancers in transdermal drug delivery using iontophoresis. *Skin Pharmacol. Appl. Skin Physiol.* **12**:326–335 (1999).
9. S. Chesnoy, D. Durand, J. Doucet, and G. Couarraze. Structural parameters involved in the permeation of propranolol HCl by iontophoresis and enhancers. *J. Control. Release* **58**:163–175 (1999).
10. K. Hirvonen, Kontturi L,. Murtomäki P, Paronen, and A. Urtti. Transdermal iontophoresis of sotalol and salicylate; the effect of skin charge and penetration enhancers. *J. Control. Release* **26**: 109–117 (1993).
11. G. L. Li, R. van der Geest, L. Chanet, E. van Zanten, M. Danhof, and J. A. Bouwstra. *In vitro* iontophoresis of R-apomorphine across human stratum corneum: The structure/transport relationship of penetration enhancement. *J. Control. Release*.
12. J. Baillie, A. T. Florence, L. R. Hume, G. T. Muirhead, and A. Rogerson. The preparation and properties of niosomes-nonionic surfactant vesicles. *J. Pharm. Pharmacol.* **37**:863–868 (1985).
13. P. M. Frederik, P. H. H. Bomans, and M. C. A. Stuart. The ultrastructure of cryo-sections and intact vitrified cells the effect of cryoprotectanta and acceleration voltage on beam induced bubbling. *Scanning Microsc. Suppl.* **5**:S43–S52 (1991).
14. J. Aungst. Structure/effect studies of fatty acid isomers as skin penetration enhancers and skin irritants. *Pharm. Res.* **6**:244–247 (1989).
15. S. K. Gupta, S. Kumar, S. Bolton, C. R. Behl, and A. W. Malick. Effect of chemical enhancers and conducting gels on iontophoretic transdermal delivery of cromolyn sodium. *J. Control. Release* **31**:229–236 (1994).
16. K. D. Peck, J. Hsu, S. K. Li, A. H. Ghanem, and W. I. Higuchi. Flux enhancement effects of ionic surfactants upon passive and elecroosmotic transdermal transport. *J. Pharm. Sci.* **87**:1161–1169 (1998).
17. W. Hoss and C. Smiley. Binding and immobilization of catecholamines by liposomes. *J. Neuroscience Res.* **3**:249–256 (1977).
18. V. Smith, R. B. Velagapudi, A. M. McLean, and R. E. Wilcox. Interactions of apomorphine with serum and tissue proteins. *J. Med. Chem. Res.* **28**:613–620 (1985).
19. G. Cevc. Transfersomes, liposomes and other lipid suspensions on the skin: Permeation enhancement, vesicle penetration, and transdermal drug delivery [review]. *Crit. Rev. Ther. Drug Carrier Syst.* **13**:257–388 (1996).
20. H. E. J. Hofland. *Vesicles as transdermal drug delivery systems*. Ph.D. Thesis, Leiden University, The Netherlands, 1992, pp. 93–132.
21. S. J. Jiang, S. M. Hwang, E. H. Choi, P. M. Elias, S. K. Ahn, and S. H. Lee. Structural and functional effects of oleic acid and iontophoresis on hairless mouse stratum corneum. *J. Invest. Dermatol.* **114**:64–70 (2000).
22. K. S. Bhatia and J. Singh. Effect of linolenic acid/ ethanol or limonene/ethanol and iontophoresis on the in vitro percutaneous absorption of LHH and ultrastructure of human epidermis. *Int. J. Pharm.* **180**:235–250 (1999).
23. K. S. Bhatia, S. Gao, T. P. Freeman, and J. Singh. Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability though porcine skin. *J. Pharm. Sci.* **86**:1011–1015 (1997).
24. N. Kalia and R. H. Guy. Interaction between penetration enhancers and iontophoresis: Effect on human skin impedance in vivo. *J. Control. Release* **44**:33–42 (1997).
25. H. E. J. Hofland, J. A. Bouwstra, J. C. Verhoef, G. Buckton, B. Z. Chowdry, M. Ponec, and H. E. Junginger. Safety aspects of non-ionic surfactant vesicles: a toxicity study related to the physicochemical characteristics of non-ionic surfactants. *J. Pharm. Pharmacol.* **44**:287–294 (1992).